# MONITORING CONTAMINANTS IN ALASKAN HARBOR SEALS

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A recent literature review on contaminants in harbor seals in Alaska, the continental U.S., and northern Europe found that data are presently insufficient to determine the current status of contaminant levels in Alaskan harbor seals (Papa and Becker 1998). What few data exist suggest levels of PCBs (polychlorinated biphenyls) and DDT residues in Alaskan harbor seals were an order of magnitude lower than those in harbor seals from the Pacific coast of the continental U.S., and two orders of magnitude less than those in seals from the Baltic Sea, southern Norway, and the Dutch Wadden Sea during the late 1980s (Papa and Becker 1998). Although levels appeared lower in Alaska than other areas, whether these levels could have produced negative biological effects has not been determined. Additionally, most data are 7 to 25 years old and cover few geographic regions (Prince William Sound, Southeast Alaska, and Kodiak). Data are particularly sparse concerning trace metals. The Alaska Department of Fish and Game (ADF&G) is currently developing and seeking collaboration with experts in the contaminants field to design a contaminants monitoring program for harbor seals in Alaska. Ideally a monitoring program would allow: (1) determination of current levels of organochlorine and heavy metal contaminants; (2) comparisons of observed contaminant levels to those in other pinniped populations world-wide, (3) comparisons of current levels to levels that produce biological effects in pinnipeds (e.g., suppressed immunity, reduced reproduction or survival), (4) tests for geographic variation in contaminant loads; (5) long-term monitoring of contaminant levels over time; and (6) preliminary health risk assessments for consumers of harbor seals.

New chemical analysis techniques allow measurement of contaminant levels in small amounts of tissues (such as blood and blubber) that can be collected from live-captured seals (Krahn *et al.* 1997). In 1999, the ADF&G collected blubber biopsies and plasma from 44 seals from Prince William Sound and 25 pups from Tugidak Island that would be suitable for these analysis methods. In 2000, whole blood was collected from 37 harbor seals from Bristol Bay. Jennifer Neale from the University of California Davis as part of her doctoral work will analyze levels of PCBs and PAHs (polycyclic aromatic hydrocarbons) in the Bristol Bay samples.

Another source for tissue samples is from subsistence-hunted seals sampled through the Alaska Native Harbor Seal Commission's biosampling program. Recommended tissues for collection from subsistence-hunted seals include blubber, muscle, liver and kidney. Metal levels should be measured in both kidney and liver to determine maximal exposure levels because, depending on the type of metal, concentrations may be higher in either tissue (Reijnders 1980, Miles *et al.* 1992, Stewart *et al.* 1999). However, harbor seal consumers prize liver but rarely use kidneys. Therefore, although metal levels in liver are of particular interest to consumers, liver samples may be limited. Working with kidney tissue will ensure a sufficient sample size and may provide a useful equation to allow prediction of liver levels from kidney levels.

Additional protocol is required for samples collected through the biosampling program to ensure data integrity and proper data analysis. Because contamination of samples from ammunition may occur, only seals shot in areas of the body remote from the tissue sampling sites should be sampled, and area/s where seals were shot and ammunition used should be recorded on the datasheet. All blubber and muscle samples should be collected from a standard region of the seal. Tissues should be collected in all five geographic regions: Southeast Alaska, Prince William Sound/Eastern Gulf of Alaska, Kodiak/Western Gulf of Alaska, Aleutians, and Bristol Bay. Age of seals (which can be determined from teeth collected during biosampling, Boveng *et al.* 1998) is often positively correlated to contaminant levels for male pinnipeds (Addison 1989, Miles *et al.* 1992), and thus should be included as a covariate in data analysis. Hunters trained by personnel from the biosampling program also measure body size and blubber thickness in the field. This information on body condition will aid interpretation of results and statistical tests, because organochlorine residue concentrations are inversely related to blubber thickness (as fat is metabolized the contaminants in the remaining tissue become more concentrated; reviewed by Addison 1989). Samples sent by hunters should be sub-sampled following protocols developed by the National Marine Mammal Tissue Bank (Becker *et al.* 1994) to prevent contamination of samples. Samples may be collected in one year and then analyzed in smaller batches (with remaining samples frozen at -80° until analyzed) to spread analysis costs over two to three years.

A large sample size is needed for precise estimates of contaminant levels. Coefficients of variation from other Alaskan harbor seal organochlorine contaminant studies ranged from 6.5% to over 200% for sample sizes ranging from 2 to 12 (calculated from Lewis 1995, Krahn *et al.* 1997). Larger sample sizes are also needed to provide enough data for statistical comparisons. For example, a preliminary power analysis demonstrated sufficient statistical power (0.80) to detect differences among five geographic regions given: a sample size of 200, use of ANOVA to analyze data, no year differences in contaminant levels between years (if samples are collected over several years), and a "medium effect" of geographic region on contaminant levels (Fig. 1). This model did not include covariates that would increase power (e.g. age and blubber thickness), and power estimates are therefore conservative. Power is dependent on effect size, or the magnitude of the difference between the null (e.g., no geographic differences) and alternative hypotheses (e.g., geographic differences present; Cohen 1988). Power will be low to detect effects of geographic region if effect size is small (conventions from Cohen, 1988 are effect size = 0.10 for small, 0.25 for medium, and 0.40 for large; Fig. 1). If significant year differences are present (ten groups in ANOVA vs. five groups), a sample size of 260 is needed for a power of 0.80 (Fig. 1). However, if year differences are present, power is improved if alpha is raised to 0.10 (Fig. 1). Therefore to ensure adequate power, an alpha level of 0.10 may be appropriate.

Chemical analysis of samples is costly, ranging from $250 to $500 per sample for organochlorines and $100 to $250 for heavy metals. To reduce costs, we suggest beginning with a pilot study to determine preliminary levels of key contaminants (20-30 PCB congeners, hexachlorobenzene, and 10-15 metals) to assist in design of a larger-scale study and to determine if a larger scale study is needed. Specifically, collection of six samples from each of the five geographic regions (total N = 30) would provide data for preliminary comparison with published levels. Because the age of seals will be known, examination of samples from only adult males will reduce variability and provide estimates of maximal contaminant levels. Loads in adult females are often lower and more variable than adult males due to dumping of organochlorines during lactation (reviewed by Addison 1989, Krahn *et al.* 1997).

Total costs for different sampling schemes considering sample sizes of 30, 50, 75 and 100 samples are shown in Table 1, based on processing costs of $700 and $450 per sample. Analysis of 30 samples for the pilot study would cost $13,500 to $22,500, compared to $90,000 to $150,000 for a full study obtaining 200 samples. Cost estimates for a 30-year monitoring program depend on the frequency of sampling (3 vs. 5 years) and the number of samples per occasion (30 to 100). Cost per year for such a program would range from $2,700 (for 30 samples every 5 years) to $25,000 (for 100 samples every 3 years; Table 1). In 2001, the ADG&F will continue to collect samples for future analyses, and search for collaborators to study levels and effects of these substances on Alaskan harbor seals.

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Table 1. Cost estimates for chemical analyses and sample processing to monitor contaminant levels in Alaskan harbor seals, based on costs per sample, number of samples collected per year, and frequency of sampling. N/Year=Number of samples collected per year. Costs are in thousands of dollars (22.5 = 22,500).

| | Cost per sample = $750 | | | | | Cost per sample = $450 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | N/ Year | | | | | N/ Year | | | | |
| Sampling scheme | 30 | 50 | 75 | 100 | 200 | 30 | 50 | 75 | 100 | 200 |
| 1 set of samples | 22.5 | 37.5 | 56.25 | 75 | 150 | 13.5 | 22.5 | 33.75 | 45 | 90 |
| Cost/year over 3 years | 7.5 | 12.5 | 18.75 | 25 | 50 | 4.5 | 7.5 | 11.25 | 15 | 30 |
| Total cost-30 year program | | | | | | | | | | |
| Every 3 years (10 sets) | 225 | 375 | 562.5 | 750 | - | 135 | 225 | 337.5 | 450 | - |
| Every 5 years (6 sets) | 135 | 225 | 337.5 | 450 | - | 81 | 135 | 202.5 | 270 | - |
| Cost difference (3 vs. 5 yrs) | 90 | 150 | 225 | 300 | - | 54 | 90 | 135 | 180 | - |
| Cost/year-30 year program | | | | | | | | | | |
| Every 3 years | 7.5 | 12.5 | 18.75 | 25 | - | 4.5 | 7.5 | 11.25 | 15 | - |
| Every 5 years | 4.5 | 7.5 | 11.25 | 15 | - | 2.7 | 4.5 | 6.75 | 9 | - |
| Cost difference (3 vs. 5 yrs) | 3 | 5 | 7.5 | 10 | - | 1.8 | 3 | 4.5 | 6 | - |

Figure 1. Power analysis for a harbor seal contaminants study based on samples collected over two years and five geographic regions. Results are shown for samples collected from five geographic regions without annual variation (five groups in ANOVA, solid line) and with annual variation over two years (ten groups in ANOVA, dashed line). (a) Effect of "effect size" on power given a sample size of 200 and alpha level of 0.05 (for explanation of effect size see text); (b) Effect of sample size given alpha level of 0.05 and effect size of 0.25 (0.25 is "medium" effect size from Cohen 1988, see text); (c) Effect of alpha level on power for ten groups (significant annual variation), given a medium effect size and sample size of 200.